REMARKS/ARGUMENTS

Applicants acknowledge with gratitude the Examiner's comments during the telephonic interviews of September 8, 2004 and September 9, 2004.

Applicants request the Examiner to consider the following remarks in light of the newly amended claims.

Status of the Claims

Claims 1-8, and 10-34 are pending. The status of the claims is as follows: Claims 3-7, 14-16, and 18-21 (original), claims 1, 2, 8, 10-13, 17, and 22-33 (currently amended), claim 9 (canceled), and claim 34 (new).

Claims 1, 3-5, 8, 14-20, 24, 27, and 33 stand rejected by the Examiner as allegedly anticipated under 35 U.S.C. §102(b) by Lo *et al.*, (WO 98/39474) and rejected under 35 U.S.C. §102(e) as allegedly anticipated by Lo *et al.*, (U.S. Pat. No.: 6,258,540). In addition, claims 1, 2, 4-5, 9-11, 13, 21-23, 27-29, and 33 stand rejected by the Examiner under 35 U.S.C. §102(b) as allegedly anticipated by Kubota *et al.*, Nature Genetics, 16, p. 16-17 (1997) (Kubota). Claim 12 stands rejected under 35 U.S.C. § 103(a) as allegedly obvious over Kubota *et al.* in view of Herman *et al.*, PNAS, 93, p. 9821-9826 (1996). Further, claims 30-32 stand rejected by the Examiner under 35 U.S.C. § 103(a) as allegedly obvious over Kubota *et al.* in view of Nuovo *et al.*, PNAS, 96, No. 22, p. 12754-12759 (1999). In addition, claims 1, 3-5, 8, 14-20, 24, 27, and 33 stand rejected as allegedly unpatentable over claims 1-7, 12-25 of U.S. Pat. No.: 6,258,540.

Applicants have amended claims 1, 2, 8, 10-13, 17, and 22-33. These amendments do not add new matter, are intended to clarify the claims, and are not intended to limit their scope. Applicants amend and cancel claims as noted without prejudice, and reserve the right to re-file the original claims to the application.

Applicants have amended Claim 1 of the instant application, from which all other claims of the application depend, to recite a method for differentiating DNA species originating from cells of different individuals, wherein the DNA species are present in a biological sample obtained from one of the individuals, the method comprising the step of detecting a methylation difference between the DNA species of the different individuals. Support for amendments of claim 1 is found in the specification. Specifically, support for "cells of different individuals" is found e.g., on page 2, lines 7-9; page 3, lines9-10; page 4, line 5; page 7, lines 10-13; page 9, lines 1-2; page 10, line 21 to page 11, line 7; page 12, lines 20-23; page 16, lines 9-24; and page 23, lines13-23. Support for "detecting a methylation difference" is found, e.g., in previous claims 2 and 9-13 and in the specification, e.g., on page 4, lines, 22, 24; page 5, lines 1-2; page 9, lines 21-23; page 12, lines 6-8; page 13, lines 5-6; page 16, lines 1-2; and page 21, lines 30-31. Support for "present in a biological sample obtained from one of the individuals" can be found, e.g., on page 2, lines 7-9; page 4, lines 4-6; page 5, lines 4-5 and 15; page 6, line 15; page 9, lines 1-2; page 10, line 2; page 11, lines 1-6; page 12, lines 5-6 and 16; page 14, line 14; page 21, lines 11-12 and previously submitted claim 37. Support for "DNA species from the different individuals" is found in claim 1

Applicants have amended claim 2 to recite "wherein the biological sample is a fluid or cellular sample or a mixture thereof." Support for this amendment is found in the specification, e.g., on page 10, lines 1-3.

Applicants have amended claims 8, 25-27, and 29. Amended claims 8, 25-27, and 29 more clearly describe Applicants' invention.

Applicants have amended claims 10-13. Amendments of claims 10-13 include deletion of the phrase "to detect a DNA methylation difference," a phrase that is now included in currently amended claim 1 from which claims 10-13 depend.

Applicants have amended claim 12 to recite "a method according to claim 1 further comprising the steps of amplifying the DNA species to generate a PCR product and sequencing the PCR product." Support for this amendment is found in the

specification, e.g., on page 15, lines 21-33; page 17, line 31 to page 18, line 14; page 18, line 25 to page 19, line 9; and page 19, line 27 -29.

Applicants have amended claims 17, 24, 28, and 30-33. Amendments of claims 17, 24, 28, and 30-33 include replacing the phrase "epigenetic" with the phrase "methylation" to more clearly describe Applicants invention.

Applicants have amended claims 22 and 23 to be dependent on claim 17.

Applicants have canceled claim 9.

Applicants have added new claim 34. Support for claim 34 is found in the specification, e.g., on page 5, line 21 and on page 12, line 22.

The Invention

To place the following remarks in their proper context, Applicants summarize their invention as to methods for distinguishing between DNA species originating from cells of different individuals present in the same biological sample, and based on epigenetic differences, such as a methylation difference. Through the detection of methylation differences, Applicants' invention allows homologous DNA species from different sources to be distinguished even when the DNA homologues have identical primary sequences.

Typically, an individual, such as a single human being, possesses within each cell one set (two copies) of homologous chromosomes. However, in the context of a pregnant female carrying a fetus, a biological sample taken from the pregnant female may not only contain cells or DNA from the female, but also cells or DNA from the fetus. Likewise, a biological sample taken from the fetus, may not only contain cells or DNA from the fetus, but also cells or DNA from the pregnant female. Thus, within a single biological sample, cells or DNAs from ***different individuals*** (different human beings) may be found. Since each individual contributes a diploid set of chromosomes, a biological sample may include four copies of any given DNA sequence (one exception being the X and Y chromosomes in male individuals). Along the same line, if the fetus

has trisomy 21, then the sample will contain two copies of chromosome 21 from the pregnant female and three copies of chromosome 21 from the fetus. These four (or five, e.g., in trisomy 21) DNA sequences may have the same primary structure (i.e., nucleotide sequence), however, they may be different in their methylation pattern, i.e., they may have an epigenetic difference.

A similar rationale applies to a transplantation recipient and an organ donor. Here, a biological sample taken from the transplantation recipient may not only contain cells and DNA from the transplantation recipient, but also cells and DNA from the organ donor. Again, within a single biological sample, cells and DNAs from ***different individuals*** (different human beings) may be found. The invention provides methods to distinguish the DNAs originating from the cells of these ***different individuals***, e.g., distinguish the DNA from the transplantation recipient from the DNA of the organ donor and likewise distinguish between the DNA of a pregnant female and a fetus. Thus, Applicants' methods claimed are different from methods in the art where the DNA of a ***single individual*** (i.e., typically only one set of homologous chromosomes) is analyzed, wherein usually a single paternally-derived sequence is distinguished from a single maternally-derived DNA species.

One method of identifying epigenetic differences is by chemically modifying the DNA sequence of a biological sample in a manner that is dependent upon the epigenetic difference, then detecting the modification. For example, methylation of bases, an epigenetic difference, can be detected by methylation-specific PCR (MSP). MSP differs from routine PCR detection of nucleic acids in that, methylation-specific PCR includes the step of treating the DNA sample with bisulfite prior to amplification. Bisulfite treatment converts unmethylated cytosyl residues to uracyl residues. Methylated cytosyl residues are unaffected by bisulfite treatment. PCR-primers that recognize methylated DNA that has not been treated with bisulfite, will not recognize the same methylated DNA after bisulfite treatment. A second set of primers specific for the DNA modified by bisulfite treatment is required to identify the modified DNA.

Therefore, the second set of primers identifies the methylation pattern of the nucleic acid, not its primary sequence. This is illustrated by the fact that two populations of methylated DNA molecules with the same primary sequence but differing methylation patterns will be recognized by different primer sets after bisulfite treatment.

Methylation differences exist between paternal and maternal chromosome sets, ***of an individual.*** However, methylation differences also exist between chromosomes ***of different individuals***, such as a pregnant female and a fetus or a transplantation recipient and an organ donor. Consequently, potentially every chromosome of an individual includes epigenetic markers that distinguish nucleic acid from one individual (e.g., the individual from whom a biological sample is taken) from that of another individual (i.e., an individual whose DNA is also present in the biological sample). Thus, the instant invention is the first to show the application of this finding to differentiating between DNA species of ***different individuals*** in a common biological sample. To emphasize this distinction again, the methods of the present invention are not simply directed to distinguish between the maternally-inherited and paternally-inherited DNA of a ***single individual***, but are directed to distinguish the DNAs of at least two ***different individuals***. This application is possible following Applicants' surprising finding that differential methylation patterns of DNA survive in a foreign host, even outside the cell.

Claim Objections

Claim 9 stands objected by the Examiner as allegedly being of improper dependent form. Applicants thank the Examiner for her helpful comments. Applicants herewith cancel claim 9.

Claim Rejections under 35 U.S.C. §102

Claims 1, 3-5, 8, 14-20, 24, 27 and 33 are not anticipated by Lo-WO.

Claims 1, 3-5, 8, 14-20, 24, 27 and 33 stand rejected by the Examiner as allegedly anticipated under 35 U.S.C. §102(b) by Lo *et al.*, (WO 98/39474) and under 35 U.S.C. §102(e) by Lo *et al.*, (U.S. Pat. No.: 6,258,540). The Examiner notes that the disclosure in both cited references is identical (referred to cumulatively hereinafter as "Lo-WO"). The Examiner also notes that Applicants' definition of "epigenetic difference" does not appear to exclude DNA concentration as a molecular difference and argues that increased quantities of a nucleic acid sequence would be a molecular difference.

For a rejection of claims under §102 to be properly founded, the Examiner must establish that a single prior art reference either expressly or inherently discloses each and every element of the claimed invention. *See, e.g. Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81 (Fed. Cir. 1986), *cert denied*, 480 U.S. 947 (1987); and *Verdegaal Bros. V. Union Oil Co. of California*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). In *Scripps Clinic & Research Found. V. Genentech, Inc.*, 18 USPQ2d 1001 (Fed. Cir. 1991), the Federal Circuit held that:

> "Invalidity for anticipation requires that **all of the elements and limitations** of the claim are found **within a single prior art reference**.... There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention." *Id.* at 1010.

Anticipation cannot be found, therefore, unless a cited reference discloses all of the elements, features or limitations of the presently claimed invention. Applicants respectfully submit that Lo-WO fails to recite all of the elements of claims 1, 3-5, 8, 14-20, 24, 27 and 33.

Lo-WO does not describe detection of a methylation difference.

The Office Action summarizes Lo-WO as teaching a method of non-invasive prenatal diagnosis including sex determination and detection of pre-eclampsia in

a mother by determining epigenetic differences between the DNA species from the mother and the fetus. Applicants respectfully disagree and rebut the rejection.

Contrary to the Office Action summary, Lo-WO does not discuss methods for differentiating between maternal and fetal DNA based on epigenetic differences, such as methylation differences. Throughout the Lo-WO reference, the authors make clear that their methods detect (I) paternally-inherited sequences which are not possessed by the mother and include (a) sequences not present in the maternal DNA (page 4, lines 9-16); (b) mutations, such as mutations in the beta globin genes (page 4, lines 17-22); paternally-inherited DNA polymorphisms or mutations (page 4, line 23 to page 5, line 2); and (II) using quantitative methods to detect different amounts of target DNA sequences, such as chromosomal aneuploidies (page 5, line 3 to page 6, line2). DNA polymorphisms and mutations are differences in the ***primary sequence*** of DNA, not epigenetic differences, such as methylation. As the Examiner has noted, Applicants definition of "epigenetic differences" expressly excludes differences in the primary nucleic acid sequence of the molecules under study.

Specifically, the Examiner refers to Example 1 (page 6) where Lo-WO describes determining the sex of a fetus by detecting the presence of a Y chromosome, using PCR with Y-specific primers. Applicants responded to this part of the Examiner's rejection in the response filed July 16, 2003. The Examiner stated in the final Office Action that she reviewed Applicants' arguments and found them convincing. Thus, no further arguments are necessary.

The Examiner also refers to Example 2 in Lo-WO as demonstrating a quantitative analysis of fetal DNA in maternal serum in aneuploid pregnancies (page 14). Further, the Examiner notes that Applicants' definition of "epigenetic difference" does not appear to exclude DNA concentration as a molecular difference and that increased molecules of nucleic acid would constitute a molecular difference.

Applicants respectfully disagree. Applicants submit that Lo-WO teaches in Example 2 the detection of fetal DNA by screening for paternally-inherited ***primary***

sequence-based DNA markers using ***standard PCR*** techniques known in the art. Those techniques are not suitable to detect epigenetic differences between DNA species, such as methylation differences. Further, in Applicants' response to a previous office, Applicants respectfully reminded the Examiner that DNA concentration is not "[a] molecular or structural difference other than the primary nucleic acid sequence," and therefore, not an ***epigenetic*** difference. The Examiner has reviewed this argument, however, deemed it not convincing.

Applicants would like to draw the Examiner's attention to the specification on page 3, lines 18-19, ("epigenetic, rather than genetic differences between the DNA species") and page 4, line 8 ("epigenetic differences in the DNA species"). Thus, it is evident that Applicants' definition refers to differences ***in the*** DNA species. A quantitative difference, such as a DNA concentration is not a qualitative difference ***in the*** DNA species. In the context of the Examiner's interpretation, the DNA species themselves would be identical, albeit different in numbers, i.e., there would be no qualitative difference ***in the*** DNA species and no alteration in the phenotype of a DNA sequence. Further, in the specification (page 3, lines 13-15), Applicants describe a skilled artisan's understanding of "epigenetic phenomena, [as] processes which ***alter the phenotype*** [of a DNA sequence] but which are not associated with changes in DNA sequence." (emphasis added)

In addition, The Random House Dictionary of the English Language refers to 'molecular [formula]' as a chemical formula that indicates the kinds of atoms and the number of each kind in a molecule of a compound and to 'structural' as pertaining to or showing the arrangement or more of attachment of the atoms which constitute a molecule of a substance.

Notwithstanding the arguments above, Applicants have amended claim 1 to recite "detecting a methylation difference" instead of "epigenetic difference." Other claims that stand rejected, namely 3-5, 8, 14-20, 24, 27, and 33 are depending on claim 1.

As the sequence differences detected using the techniques of Lo-WO fall outside the methods of the present invention and outside of "detecting a methylation difference," Lo-WO fails to teach all of the elements of Applicants invention. Applicants respectfully request withdrawal of the rejection of record.

Claims 1, 2, 4-5, 9-11, 13, 21-23, 27-29 and 33 are not anticipated by Kubota.

Claims 1, 2, 4-5, 9-11, 13, 21-23, 27-29 and 33 stand rejected by the Examiner under 35 U.S.C. §102(b) as allegedly anticipated by Kubota *et al.*, Nature Genetics, 16, p. 16-17 (1997) (Kubota). Applicants respectfully rebut the rejection.

Kubota is described as "teaching" genomic imprinting, in the form of differential methylation between the maternal homologue and the paternal homologue plays an important role in Prader-Willi syndrome (PWS) and Angelman syndrome (AS) (citing to page 16, col. 1). Kubota is said to describe diagnosing the diseases by showing an affected ***individual*** lacks either a 100-bp PCR product (PWS) or a 174-bp PCR product (AS) present in a normal ***individual***. This description allegedly "teaches" a method of differentiating DNA species from different individuals in a blood sample by determining differing methylation patterns between the DNA species. Applicants respectfully disagree.

Kubota discusses a method of diagnosing diseases by taking advantage of methylation differences in the ***homologues*** of maternally-inherited and paternally-inherited genes taken ***from the same individual***, not a method for determining differing methylation patterns between DNA species from ***different individuals***, as recited in Applicants' claims.

Kubota identifies PWS and AS as genetic diseases, and describes a method of diagnosis that identifies aberrant structure or methylation of chromosome 15 (see page 16, particularly fig 1). The Examiner acknowledges that the diagnosis identifies the 100-bp and 174-bp PCR products as originating from ***homologous***

chromosomes (maternal and paternal), and that only "normal" ***individuals*** display both the 174-bp and 100-bp PCR products. Diseased ***individuals*** lack one or both MSP products. (see Kubota, p16, cols 2 and 3).

Applicants respectfully point out that every ***individual*** possesses a diploid set of ***homologous chromosomes*** (one paternally-inherited and one maternally-inherited). Therefore, in referring to parental and maternal chromosomes, Kubota is discussing chromosome endogenous to ***one individual***, and not describing differentiating DNA species from ***different individuals***. ***Nowhere*** does Kubota discuss the use of differing methylation patterns as a means for differentiating DNA species from ***different individuals***, as recited in claim 1 of the instant application.

Notwithstanding the arguments above, Applicants have amended claim 1 as described herein. According to claim 1, the DNA species to be analyzed using the methods of the present invention originate from ***cells of different individuals*** (i.e., at least from the cells of ***two individuals***, such as pregnant female and fetus or transplantation recipient and organ donor). Because typically every individual's cell possesses a pair of homologous chromosomes, the DNA species from those at least two different individuals, inherently, comprise ***four homologous DNA strands***. In trisomy 21, even five homologous DNA strands of chromosome 21 are present. In addition, the four homologous DNA strands are present in a biological sample (i.e., in a single or common biological sample). Further, this biological sample is obtained from one of the individuals whose DNA species is to be analyzed. Finally, a methylation difference is detected between the DNA species of the individual from whom the biological sample is obtained and the DNA of the other individual whose cells or DNA were included in the biological sample.

Kubota simply does not anticipate a method for differentiating DNA species originating from cells of different individuals, wherein the DNA species are present in a biological sample obtained from one of the individuals, the method

comprising the step of detecting a methylation difference between the DNA species from the different individuals.

Kubota simply teaches the analysis of a maternal homologue and a paternal homologue within a single individual. Thus, based on the teachings of Kubota, ***only two*** differently methylated DNA sequences may be identified - simply because there are only two homologous DNA strands in Kubota's samples. However, in Applicants' invention four homologous DNA strands are in the biological sample. As such, ***four*** differently methylated DNA sequences may be identified, namely the maternal and paternal homologues of individual I (e.g., the pregnant female or the transplantation recipient) ***and*** the maternal and paternal homologues of individual II (e.g., the fetus or organ donor). Likewise, in trisomy 21, five homologous strands of chromosome 21 can be analyzed. Thus, Applicants' invention is suitable to analyze two maternally-inherited DNA homologues in a single biological sample taken from one individual, i.e., more specifically to analyze maternally-inherited DNA originating from cells of individual I ***and*** maternally-inherited DNA originating from cells of individual II in one sample. For example, Figure 4 and page 20, line 26 to page 21, line 18 of Applicants' application demonstrates the detection of unmethylated (maternally-inherited) fetal DNA (i.e., individual 1) in maternal plasma, i.e., also containing another maternally-inherited DNA homologue, namely that of the mother (i.e., individual II). The same principle is shown in Applicants' EXAMPLE 1, where male marrow transplantation recipients received bone marrow from female donors. In stark contrast to Applicants' invention, Kubota teaches the analysis of ***only one*** maternally-derived homologue and ***only one*** paternally-derived homologue.

Therefore, Kubota cannot anticipate Applicants' claims. Applicants respectfully request withdrawal of the rejection of record.

Claim Rejections under 35 U.S.C. §103(a)

Combining Kubota and Herman does not render Claim 12 obvious.

The Examiner has rejected claim 12 under 35 U.S.C. § 103(a) as allegedly obvious over Kubota *et al* (Kubota) in view of Herman *et al.* (Herman), PNAS, 93, p. 9821-9826 (1996). Applicants respectfully traverse.

Kubota is described in the final Office Action as teaching a method of differentiating DNA species from different individuals in a biological sample, namely blood, amniotic fluid or chorionic villus, by determining epigenetic differences, namely methylation, between the DNA species (page 17, col. 1).

Herman is described as teaching that following chemical modification of cytosine to uracil by bisulfite treatment, the altered DNA may be amplified and sequenced to provide detailed information within the amplified region of the methylation status of all CpG sites. The Office Action concludes that it would have been *prima facie* obvious to sequence CpG sites described in Kubota, as sequencing is "an equivalent means of determining methylation status," and provides "detailed information... of the methylation status of all CpG sites." Applicants respectfully disagree.

Establishing a *prima facie* case for obviousness under §103 requires the Examiner show, *inter alia*:

(1) The prior art references teach or suggest all claim limitations of the rejected claim(s). *In re Royka*, 180 USPQ 580 (CCPA 1974); and MPEP §2143.03.

(2) The existence of some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine the reference teachings. *In re Fine*, 5 USPQ2d 1596 (Fed. Cir. 1988).

(3) A reasonable expectation of success in combining the references. This must be found in the prior art, and not in the applicants disclosure. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991).

The combination fails to teach all elements of the present invention.

Applicants note that the Examiner did not respond to Applicants' arguments forwarded in response to the previous Office Action (submitted July 14, 2003)

and demonstrating that the prior art references cited do not teach or suggest all claim limitations of the rejected claim(s).

As discussed above in response to the anticipation rejection, Kubota describes a method of detecting aberrant methylation associated with PWS and AS in ***a single individual***. Kubota teaches the analysis of ***only one*** maternally-derived homologue and ***only one*** paternally-derived homologue. Kubota does not suggest a method for differentiating DNA species originating from cells of different individuals, wherein the DNA species are present in a biological sample obtained from one of the individuals, the method comprising the step of detecting a methylation difference between the DNA species from the different individuals as recited in Applicants' claims.

Herman discusses the use of MSP as a tool in mapping DNA methylation patterns to better understand biological processes such as regulation of imprinted genes, X chromosome inactivation and tumor suppressor gene silencing in human cancer. Herman does not suggest a method for differentiating DNA species originating from cells of different individuals, wherein the DNA species are present in a biological sample obtained from one of the individuals, the method comprising the step of detecting a methylation difference between the DNA species from the different individuals as recited in Applicants' claims.

As the combination of references suggested in the Office Action ***fails to provide all of the elements*** of Applicants' claimed invention, a *prima facie* case of obviousness has not been set forth. Therefore, Applicants respectfully request the rejection to claim 12 be withdrawn.

No motivation to make the suggested combination

Assuming *arguendo* that the suggested combination possessed all of the elements of Applicants invention, the obviousness rejection would still be improper as there is no motivation to make the suggested combination to arrive at Applicants invention.

Applicants respectfully point out Kubota's reasoning for choosing MSP as the analytical method of his assay was because:

> "[MSP] provides a reliable diagnostic method for all PWS and AS patients who show abnormal methylation at SNRPN. The ***major advantage*** of [MSP] is the ***rapidity*** of a PCR-based assay compared with a Southern Blot assay. This may have ***important implications*** for early diagnosis/management of hypotonic infants who may be suspected of having PWS, and for prenatal diagnosis." (Kubota col. 2, p. 17.)

The Office Action proposes modifying the Kubota method by detecting methylation patterns by DNA sequencing, as allegedly taught by Herman, rather than with MSP as chosen by Kubota. Applicants respectfully suggest that the proposed combination, if workable, would frustrate rapid diagnosis of the condition ***sought by Kubota*** without providing any additional meaningful information.

The Office Action looks for support in Herman's statement that:

> "The only technique that can provide more direct analysis than MSP for most CpG sites within a defined region is ***genomic sequencing***" (page 9825 col. 2)

However, in the passage ***immediately*** after this statement Herman summarizes why ***DNA sequencing is inferior*** to MSP for mapping methylation patterns:

> "However, MSP can provide ***similar information*** and has the following advantages. First, MSP is much ***simpler*** and ***requires less time*** than genomic sequencing, with a typical PCR and gel analysis taking 4-6hr. In contrast, for genomic sequencing, amplification, cloning, and subsequent sequencing may take days. Second, MSP ***avoids*** the use of expensive sequencing reagents and the use of radioactivity. ... Third, the use of PCR as the step to distinguish methylated from unmethylated DNA in MSP allows for a significant ***increase in the sensitivity*** of methylation detection." (Herman at 9825-26.)

Herman then presents statistics illustrating the ***inferiority of DNA sequencing*** in determining methylation status of a CpG island, compared to MSP, concluding:

> "In summary, MSP is a ***simple, sensitive, and specific*** method for determining the methylation status of virtually any CpG-rich region." (Herman at 9826, col. 1)

In view of Herman's ***disparaging remarks*** regarding DNA sequencing in these types of assays, one of skill could not find motivation in the reference and in fact would have no reasonable expectation of success in making the suggested combination.

Moreover, as Herman describes DNA sequencing as an inferior analysis to MSP contradicting the Office Action conclusion that the two methods are "equivalent," and Kubota chooses MSP over sequencing, Applicants respectfully suggest that the references are combined through impermissible hindsight, as the only motivation for combination resides in Applicants disclosure.

Thus, to more clearly describe Applicants' invention, Applicants have amended claim 12 to recite "a method according to claim 1 further comprising the steps of amplifying the DNA species to generate a PCR product and sequencing the PCR product."

As the references suggested in the Office Action ***fail to motivate to make the suggested combination*** of Applicants' claimed invention, a *prima facie* case of obviousness has not been set forth. Therefore, Applicants respectfully request the rejection to claim 12 be withdrawn.

Combining Kubota and Nuovo does not render claims 30-32 obvious.

Claims 30-32 stand rejected by the Examiner under 35 U.S.C. § 103(a) as obvious over Kubota *et al.* (Kubota)in view of Nuovo *et al.* (Nuovo), PNAS, 96, No. 22, p. 12754-12759 (1999).

The Office Action summarizes Kubota as above, adding that Kubota's method analyzes DNA from pregnant women to detect the presence of PWS and AS. According to the Examiner, Kubota does not specifically teach using methylation specific polymerase chain reaction (PCR) in situ to detect methylation.

Nuovo is described as teaching *in situ* detection of methylation using an *in situ* methylation-specific PCR (MSP-ISH). According to the Office Action, Nuovo applies MSP-ISH to tracing the evolution of cell populations harboring hypermethylation-associated inactivation, and identifying changes in specific cell types during embryonic development.

The Examiner concludes that it is obvious to modify and improve the method of Kubota to detect the presence of PWS and AS in pregnant women. The Examiner further believes that modifying Kubota to include MSP-ISH will provide additional information regarding the precise timing of DNA methylation and change in specific cell types during embryonic development, as suggested by Nuovo.

Applicants respectfully disagree. The legal standard for establishing a *prima facie* case for obviousness under §103 have been addressed above.

The combination fails to teach all elements of the present invention.

Applicants note that the Examiner did not respond to Applicants' arguments forwarded in response to the previous Office Action (submitted July 14, 2003) and demonstrating that the prior art references cited do not teach or suggest all claim limitations of the rejected claim(s).

Neither Kubota nor Nuovo alone or in combination teach all elements of Applicants' claimed invention. They do not suggest a method for differentiating DNA species originating from cells of different individuals, wherein the DNA species are present in a biological sample obtained from one of the individuals, the method comprising the step of detecting a methylation difference between the DNA species from the different individuals as recited in Applicants' claims. Therefore, the combination cannot sustain a *prima facie* case of obviousness.

Claims 30-32 are dependent from claim 1 and incorporate all the elements and limitations of the parent claim(s). 35 U.S.C. §112 paragraph 4; and MPEP §608.01(n)(III). Claims 30-32 are therefore drawn to a method for differentiating DNA species originating from cells of different individuals, wherein the DNA species are

present in a biological sample obtained from one of the individuals, the method comprising the step of detecting a methylation difference between the DNA species from the different individuals.

As described above, Kubota discusses a test for ***genetic aberrations*** associated with PWS and AS ***in single individuals***, i.e., the analysis of ***one*** maternally-derived homologue and ***one*** paternally-derived homologue in a single individual. Thus, Kubota does not discuss differentiating between DNA species from different individuals in a biological sample, i.e., the analysis of ***two*** maternally-derived homologues and ***two*** paternally-derived homologues in a single individual. Nor does Kubota discuss differentiating between five homologous DNA species in trisomy 21.

Similarly, Nuovo generally describes a method of charting changes in DNA methylation patterns during tumor progression in an individual. Embryogenic study is mentioned speculatively as another possible use of the Nuovo method. Regardless Nuovo, like Kubota, provides no description of a method for differentiating DNA species originating from cells of different individuals, wherein the DNA species are present in a biological sample obtained from one of the individuals, the method comprising the step of detecting a methylation difference between the DNA species from the different individuals, as recited in claim 1 from which claims 30-32 depend.

Further, Nuovo does not compensate for the deficiencies previously mentioned and found in Kubota and the proposed combination of Kubota and Nuova fails to teach each element as found in the Applicants' claims. As such, the Office Action fails to set forth a *prima facie* case of obviousness based on the cited references. Therefore, Applicants respectfully request withdrawal of the rejection of record.

No motivation to make the suggested combination

Assuming *arguendo* that the suggested combination possessed all of the elements of Applicants invention, the obviousness rejection would still be improper as there is no motivation to make the suggested combination to arrive at Applicants invention.

As explained above, Kubota describes a simple, quick diagnostic method for identifying the genetic disorders PWS and AS in a single individual. Kubota believes:

> "This may have important implications for early diagnosis/management of hypotonic infants who may be suspected of having PWS, and for prenatal diagnosis." (Kubota col. 2, p. 17.)

Nuovo discusses methods of identifying and tracking hypermethylation of certain genes *in situ* using archived malignant cell preparations taken from an individual. These methods allow for the dissection of hypermethylation events in the progression of neoplastic tumors, and are postulated as being useful for the diagnosis of certain genetic disorders. (see page 12759 generally).

Nuovo discusses the MSP-ISH procedure on pp. 12754 -12755. The procedure includes making multiple sections from the tissue under study, performing MSP-ISH on each section, followed by microscopic analysis.

Again, assuming *arguendo* the combined references taught each element of Applicants claimed invention, Applicants respectfully suggest that proposed modification defeats the stated goal of Kubota, providing nothing to aid or confirm a simple, quick diagnosis. For example, the Nuovo method typically requires manual microscopic examination of samples that are time-consuming to conduct. Nuovo provides no suggestion of ways to avoid manual examination, and absent microscopic examination it is not clear how the combination would provide the advantage suggested in the Office Action as motivation, i.e., to "provide additional information regarding the precise timing of DNA methylation and change in specific cell types during embryonic development."

As the references suggested in the Office Action ***fail to motivate to make the suggested combination*** of Applicants' claimed invention, a *prima facie* case of obviousness has not been set forth. Therefore, Applicants respectfully request withdrawal of the rejection of record.

Obviousness-type Double Patenting

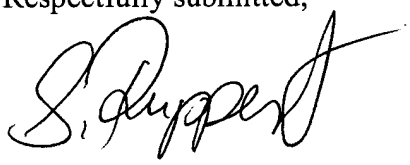
Claims 1, 3-5, 8, 14-20, 24, 27 and 33 stand rejected as allegedly unpatentable over claims 1-7, and 12-25 of U.S. Pat. No.: 6,258,540. This is essentially the same rejection as the one made by the Examiner under 35 U.S.C. § 102(b) and § 102(e) rejection based on this patent above. Applicants therefore respond in the same manner here, as above and respectfully request withdrawal of the rejection of record.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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